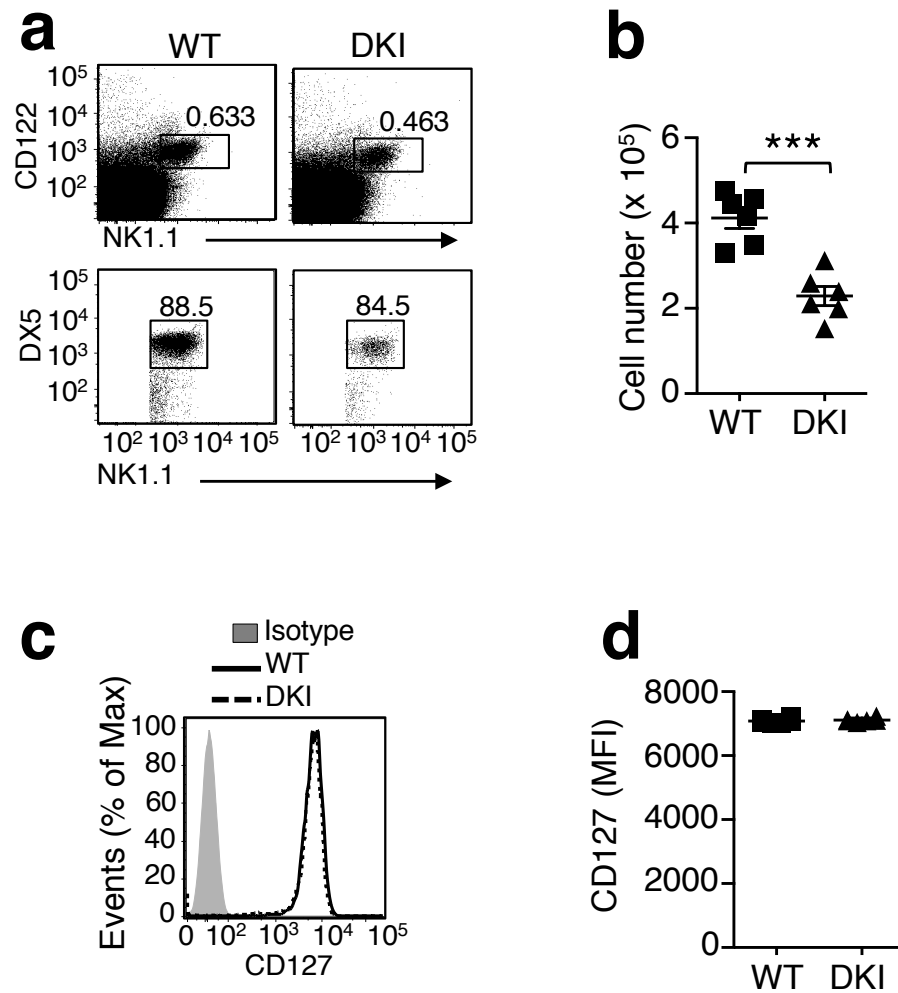


# Supplementary Figure 1

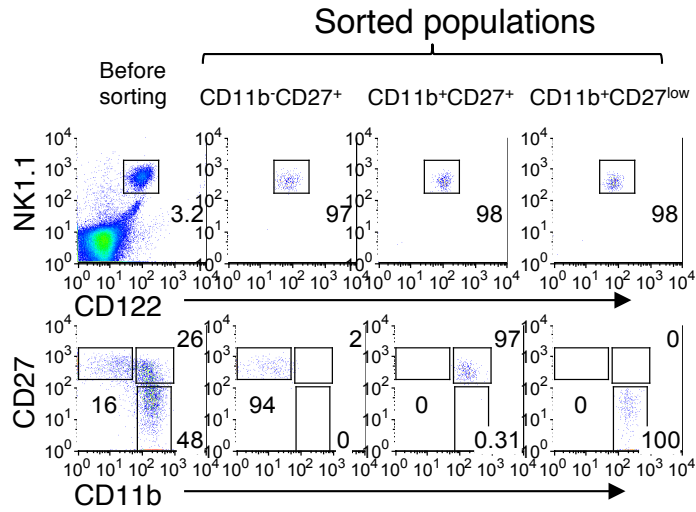


**Supplementary Figure 1. Decreased thymic NK cells in *Stat5* DKI mice.** (a) Flow cytometric analysis of total thymic NK cells (lin<sup>-</sup>[TCR $\beta$ CD3CD4CD8CD19Ter119]<sup>-</sup> CD122<sup>+</sup>) (upper panels) and lin<sup>-</sup>CD122<sup>+</sup>NK1.1<sup>+</sup>DX5<sup>+</sup> mature NK cells (lower panels) in WT and *Stat5* DKI thymus. (b) Total thymic NK cell numbers from 6 WT (black squares) and 6 *Stat5* DKI (black triangles) mice. The experiment was performed twice, each with 3 WT and 3 DKI mice; shown are representative mice. The differences in the thymic NK cell numbers between WT and *Stat5* DKI mice were significant (Multiple *t* test, *p* < 0.001). (c) Representative flow cytometric profiles for CD127 expression on *Stat5* DKI (dotted line) and WT (solid line) thymic NK cells. (d) Summary of CD127 levels (MFI) between WT (black squares) and DKI (black triangles) thymic NK cells. Error bars are means  $\pm$  SEM.

# Supplementary Figure 2

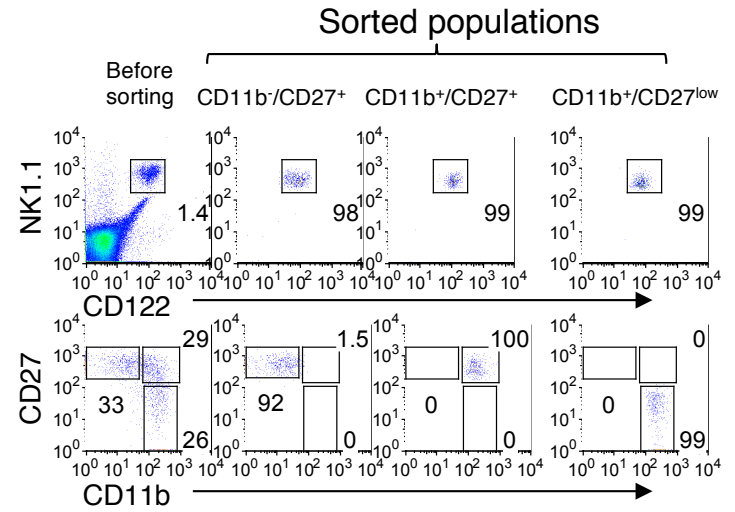
**a**

WT CD3<sup>-</sup> cells

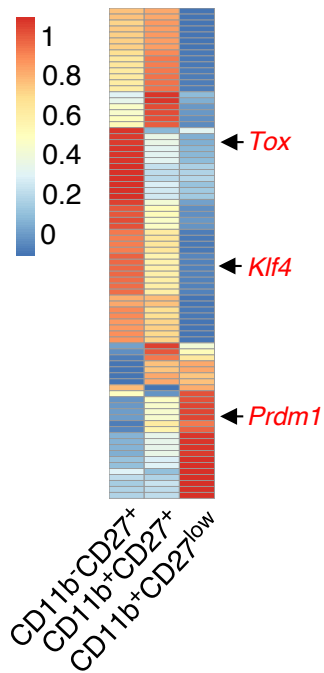


**b**

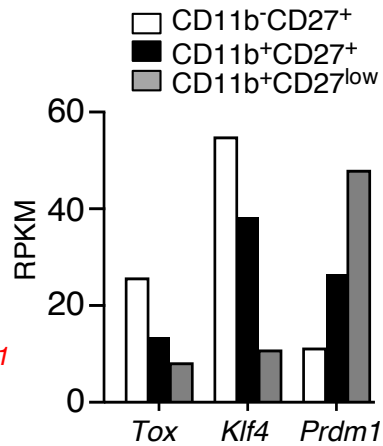
DKI CD3<sup>-</sup> cells



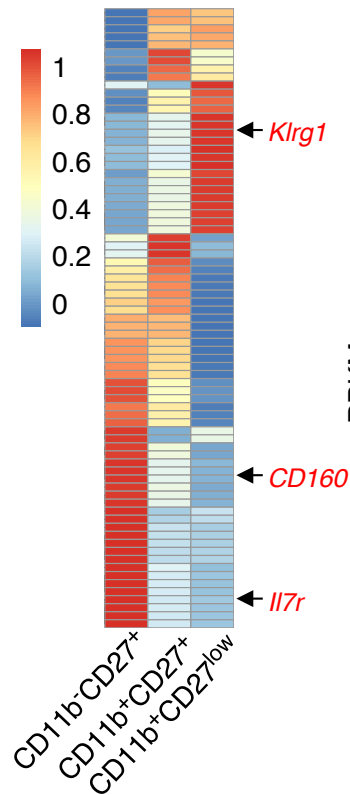
**c**



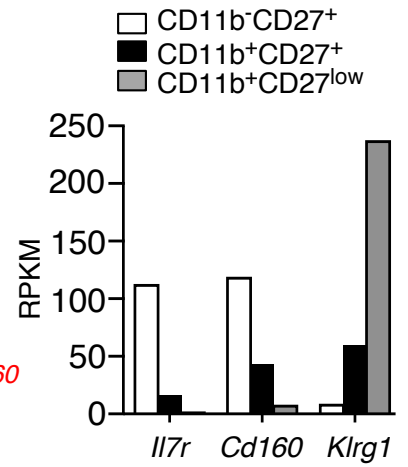
**d**



**e**

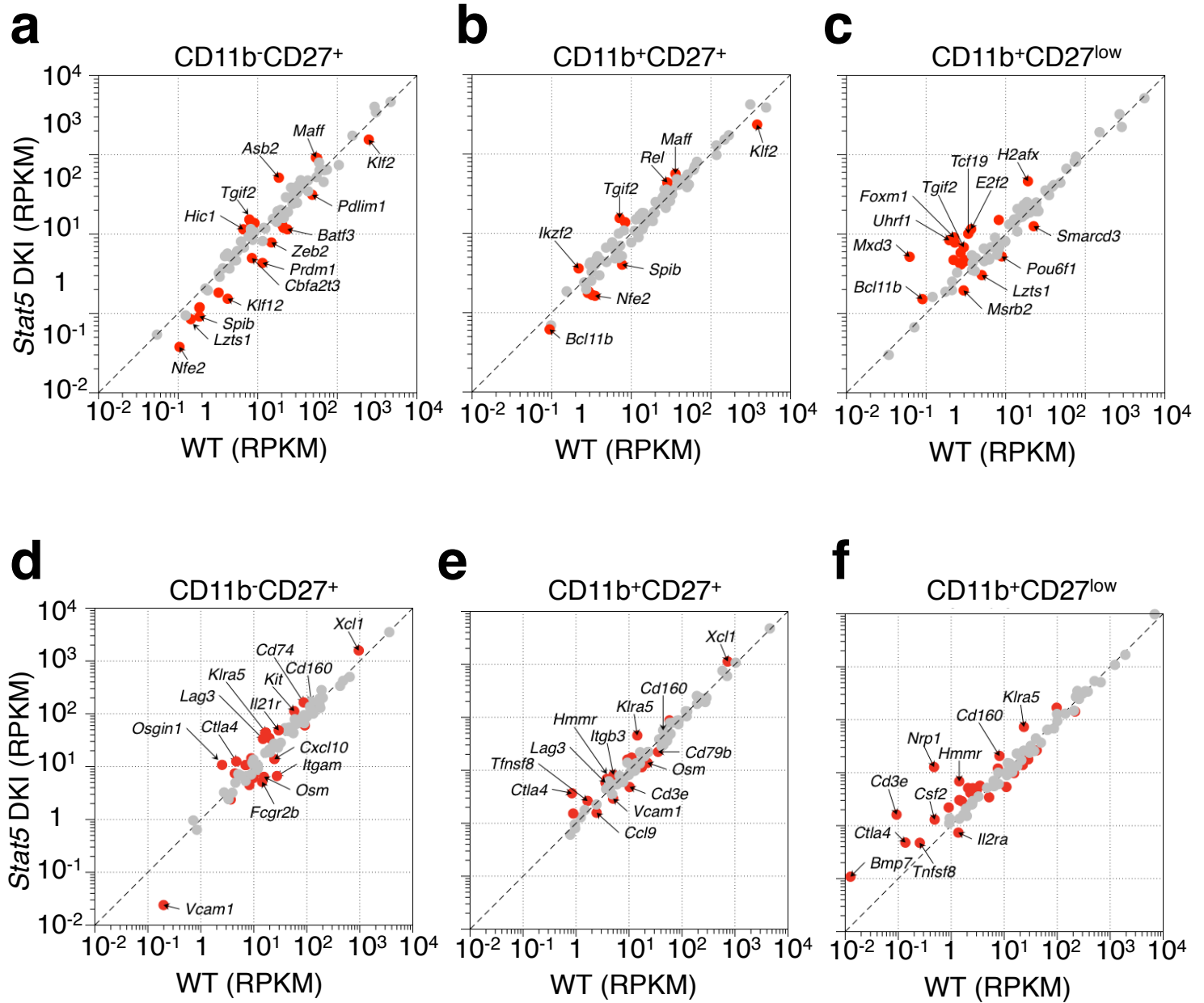


**f**



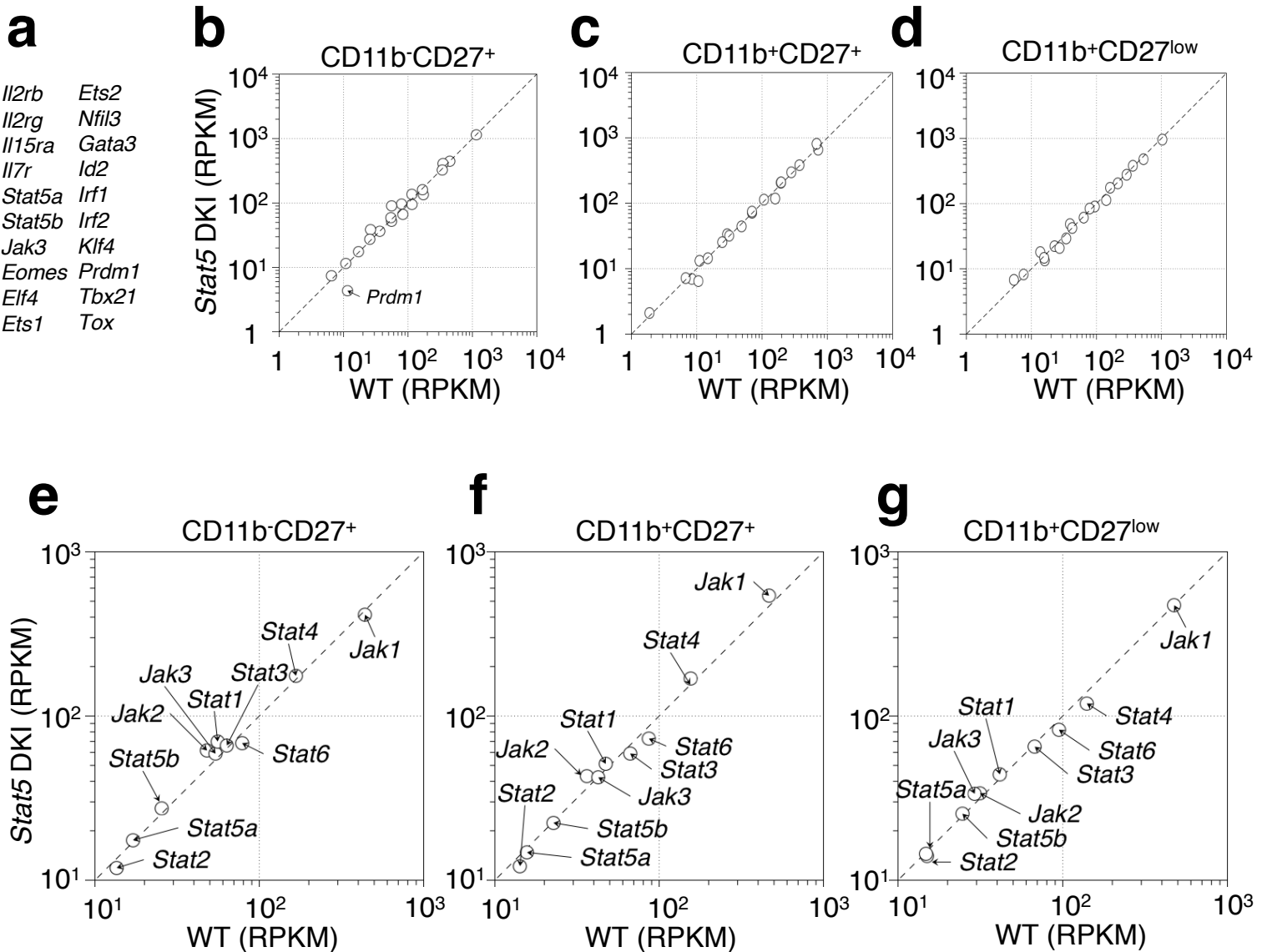
**Supplementary Figure 2. Differentially expressed genes encoding transcription factors and receptors in WT CD11b<sup>-</sup>CD27<sup>+</sup>, CD11b<sup>+</sup>CD27<sup>+</sup>, and CD11b<sup>+</sup>CD27<sup>low</sup> subpopulations of NK cells.** (a) Flow cytometric profiles of splenic NK cells before and after sorting from WT mice. (b) Flow cytometric profiles of splenic NK cells before and after sorting from *Stat5* DK1 mice. (c) Heat maps of mRNAs for transcription factors differentially expressed in WT NK subpopulations. (d) Expression (RPKM) shown for three transcription factors (*Tox*, *Klf4*, and *Prdm1*) known to be important for NK cell development and/or maturation. (e) Heat maps of mRNAs for cytokines, chemokines, their receptors, NK receptors, and other receptors differentially expressed in WT NK subpopulations, (f) Expression (RPKM) shown for a cytokine receptor (*Il7r*) and two NK receptors (*Cd160* and *Klrg1*).

# Supplementary Figure 3



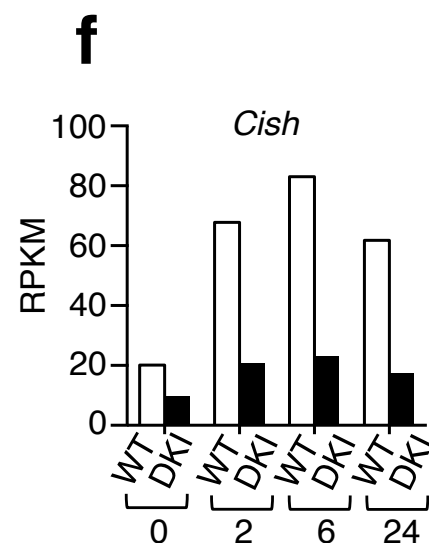
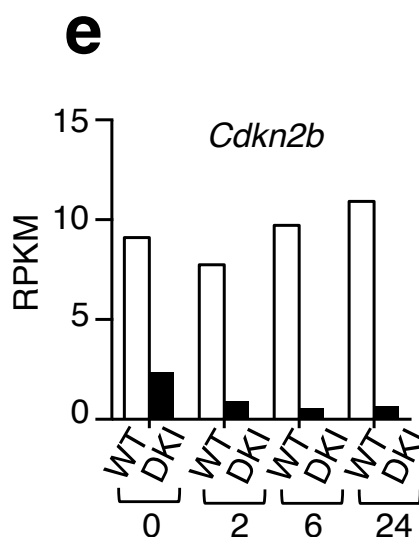
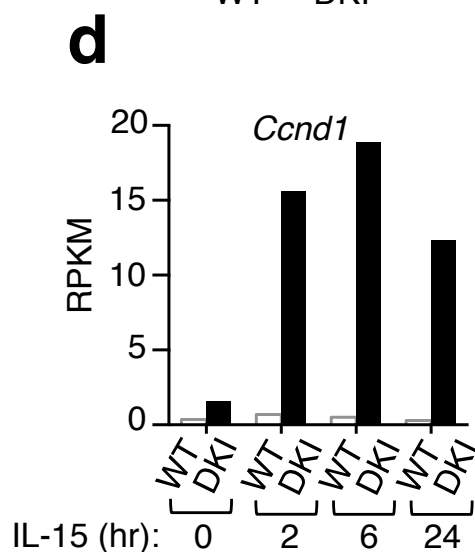
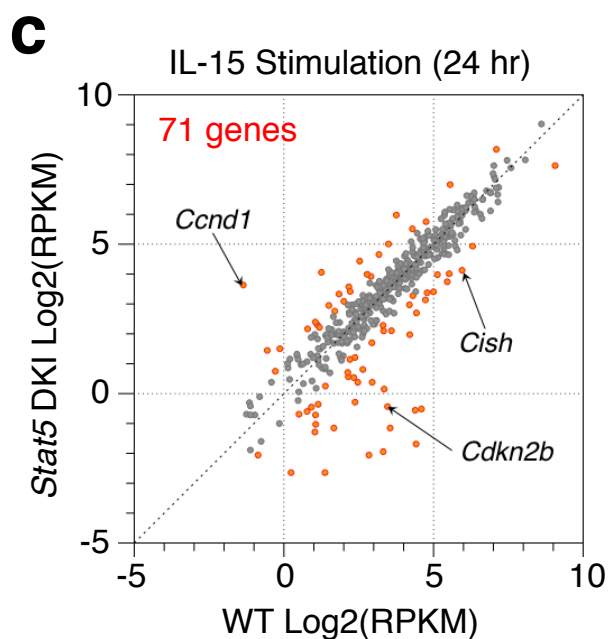
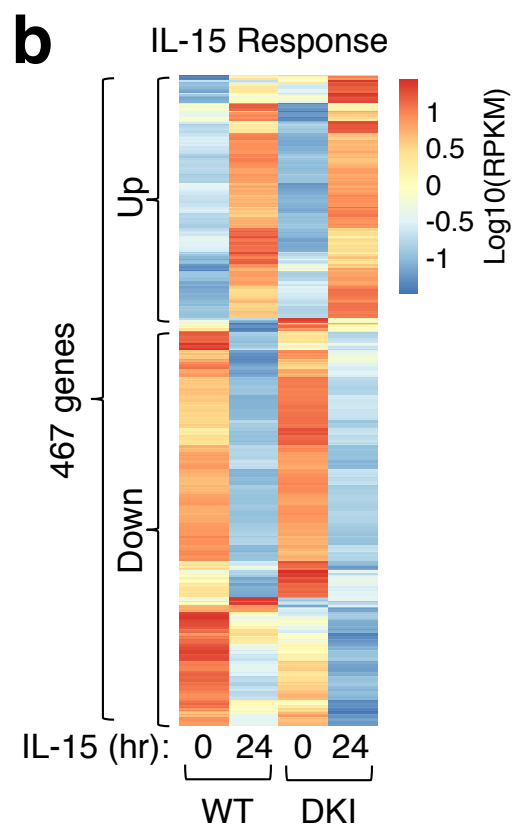
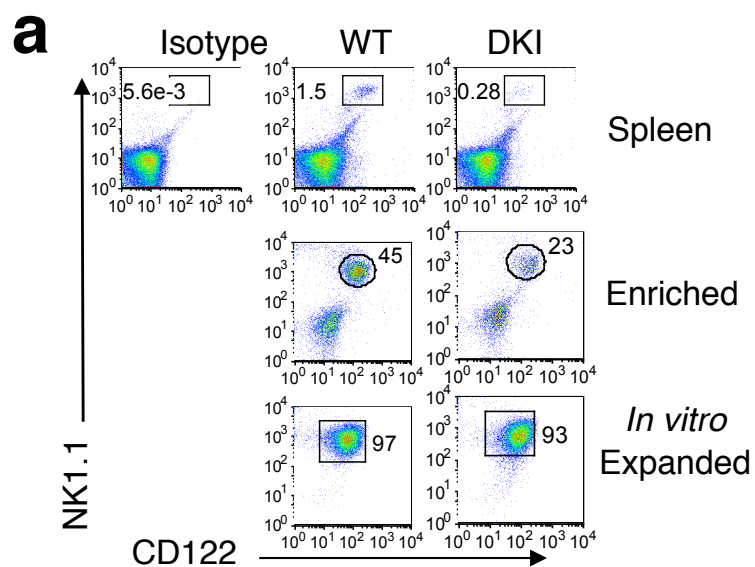
**Supplementary Figure 3. Altered expression of genes encoding transcription factors and receptors in CD11b<sup>-</sup>CD27<sup>+</sup>, CD11b<sup>+</sup>CD27<sup>+</sup>, and CD11b<sup>+</sup>CD27<sup>low</sup> subpopulations of *Stat5* DKI NK cells.** (a-c) Scatter plots of genes encoding transcription factors whose expression were altered (in solid red circles) in CD11b<sup>-</sup>CD27<sup>+</sup> (a), CD11b<sup>+</sup>CD27<sup>+</sup> (b), and CD11b<sup>+</sup>CD27<sup>low</sup> (c) subpopulations of *Stat5* DKI NK cells. (d-f) Scatter plots of genes encoding receptors whose expression were altered (in solid red circles) in CD11b<sup>-</sup>CD27<sup>+</sup> (d), CD11b<sup>+</sup>CD27<sup>+</sup> (e), and CD11b<sup>+</sup>CD27<sup>low</sup> (f) subpopulations of *Stat5* DKI NK cells.

# Supplementary Figure 4



**Supplementary Figure 4. Expression profile of genes that are known to be critical for NK cell development and those encoding JAK kinases and STAT proteins. (a)** List of genes that are known to be critical for NK cell development and maturation. **(b-d)** Scatter Plots showing generally similar expression of the genes in panel **a** in WT and *Stat5* DKI CD11b<sup>-</sup>CD27<sup>+</sup> **(h)**, CD11b<sup>+</sup>CD27<sup>+</sup> **(i)**, and CD11b<sup>+</sup>CD27<sup>low</sup> **(j)** NK cells. **(e-g)** Scatter plots of genes encoding JAK kinases and STAT proteins in CD11b<sup>-</sup>CD27<sup>+</sup> **(e)**, CD11b<sup>+</sup>CD27<sup>+</sup> **(f)**, and CD11b<sup>+</sup>CD27<sup>low</sup> **(g)** subpopulations of *Stat5* DKI and WT NK cells.

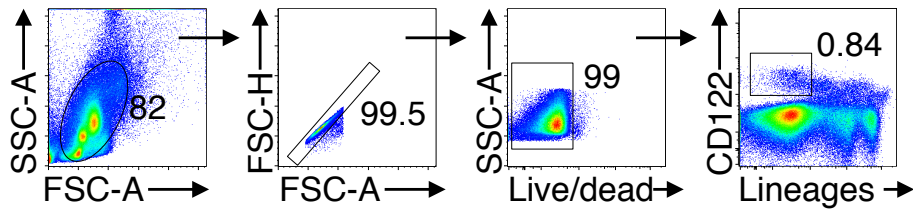
Supplementary Figure 5



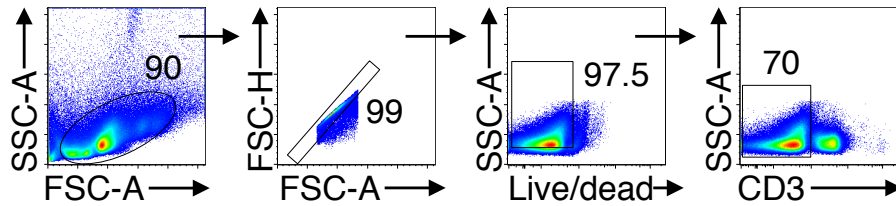
**Supplementary Figure 5. Gene expression profiles of WT and *Stat5* DKI NK cells in response to IL-15 stimulation.** (a) Splenic NK cells were isolated and purified from WT and *Stat5* DKI mice. NK cells (CD3<sup>-</sup>CD122<sup>+</sup>NK1.1<sup>+</sup>; top and middle rows) were expanded *in vitro* by culturing with IL-15 for 6 days (bottom row). (b) Heat map showing genes regulated by IL-15 in WT and *Stat5* DKI NK cells, with 467 genes up- or down-regulated in WT NK cells in response to IL-15, as indicated by black brackets on the left. The expression of some genes was altered in *Stat5* DKI NK cells. (c) Scatter plot showing 71 genes (red circles) whose expression was altered in *Stat5* DKI NK cells stimulated by IL-15 for 24 hr. (d) Bar graph showing that the expression of *Ccnd1* was not regulated by IL-15 in WT NK cells but was significantly altered in *Stat5* DKI NK cells. Expression is indicated by RPKM. (e) Bar graph showing that the expression of *Cdkn2b* was not regulated by IL-15 in WT NK cells but was significantly altered in *Stat5* DKI NK cells. Expression is indicated by RPKM. (f) Bar graph showing that the expression of *Cish* was potently induced by IL-15 in WT NK cells but not in *Stat5* DKI NK cells. Expression is indicated by RPKM.

## Supplementary Figure 6

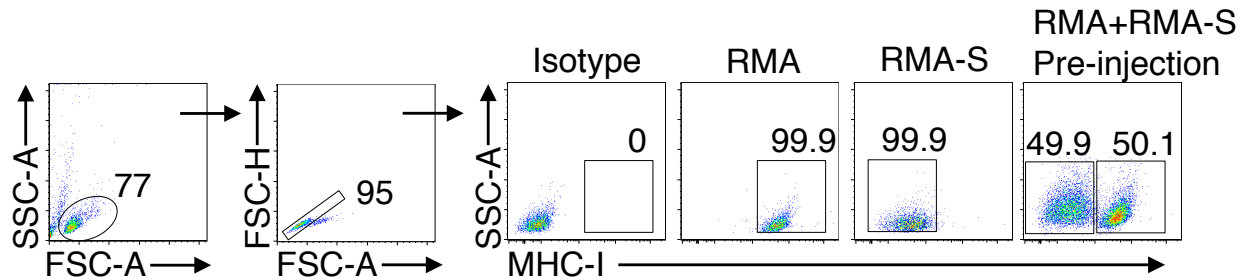
### a Bone marrow NK cells



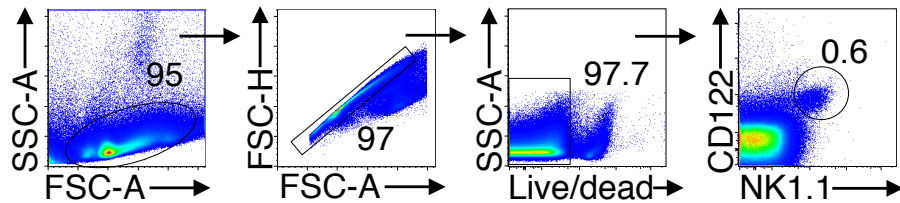
### b Spleen NK cells



### c RMA & RMA-S



### d Thymic NK cells



**Supplementary Figure 6.** Flow cytometry gating and sorting strategies. **(a)** Gating strategies for flow cytometric analysis of all bone marrow NK cells (Fig. 1b, 1d and 5c). **(b)** Gating strategies for flow cytometric analysis of all spleen NK cells (Fig. 1g, 5a, 5d, 6d, and Supplementary Fig. 2 and 5). **(c)** Gating strategy for Fig. 3c. **(d)** Gating strategies for Supplementary Fig. 1.